

Identification of relationships among EDIL3, angiogenesis and inflammation in colorectal cancer: In-silico analysis

EDIL3 in colorectal cancer

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Abstract

Aim: The relationship between inflammation and the development of colorectal adenocarcinoma has been described for a number of years. In this cancer, chronic inflammation causes cellular damage and DNA mutations responsible for the proliferation of transformed cells. Epidermal growth factor-like repeats and discoidin I-like domains 3 (EDIL3/DEL-1) were anti-inflammatory protein, especially in epithelial cells. Our study, it was aimed to clarify the link of among IL-17A, EDIL3/DEL1, and the main angiogenic factor VEGFA/B in colorectal cancer.

Materials and Methods: We investigated EDIL3, IL-17, and VEGFA/B mutations profile and levels of m-RNA expressions in 594 colorectal cancer patients using bioinformatics analysis for the revealing relationship among these molecules. Additionally, PolyPhen-2 and SNAP tools were used to predict and confirm the pathogenicity of the detected mutations.

Results: In colorectal cancer patients, the IL-17 and EDIL3 expressions were inversely correlated but the low correlation coefficient was statistically significant. EDIL3 and VEGFB m-RNA levels were found to be statistically significantly lower in the patient group than in the healthy group. Furthermore, 10 mutations were identified in the EDIL3. The study predicted the potential pathological effect of eight mutations in the EDIL3.

Discussion: Low expression of EDIL3 might contribute to anti-inflammation, supporting extravasation of leukocytes to inflammatory area by increased IL-17 expression. Although further studies to clarify the mechanisms explaining the role of EDIL3 in tumor angiogenesis in terms of VEGF gene, and the interaction between IL-17 and EDIL3 are required, the results indicate that EDIL3 may be a good novel molecular target gene in inflammatory pathways for colorectal cancer.

Keywords

EDIL3/DEL-1; Colon cancer; Inflammation; Mutation; Gene expression; Angiogenesis

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Introduction

Cancer is one of the most important health problems today, and because of the high death risk and its widespread prevalence, it has become a public health disease [1]. Colorectal cancer (CRC) is the third most common cancer worldwide and has a complex etiology consisting of environmental, genetic, epigenetic factors, and chance [2]. The World Health Organization predicts that colon cancer, which in 2018 has already affected 1.80 million cases, will be the leading cause of death worldwide [3]. This type of cancer is associated with elevated levels of biomolecules of the immune system in the tumor microenvironment, which may lead to changes in the tumor characteristic, such as proliferation, invasion, vascularisation, and metastasis [4]. In this study, we examine what bioinformatic analysis reveals about link among IL-17A, EDIL3/DEL1, and the main angiogenic factor VEGFA/B in colorectal cancer.

The first point is that EDIL3/DEL1 is included in the stimulation of angiogenesis, cell adhesion, and cell migration signaling pathways [5,6]. This protein is a molecular marker for embryonic endothelial cells and is rarely expressed after birth. However, it has been demonstrated that EDIL3/DEL1 was expressed in numerous types of cancer including colon cancer, bladder cancer, hepatocellular carcinoma [5-9]. Overexpressed EDIL3/DEL1 expression causes tumor growth and metastasis through stimulation of neovascularization [7-9]. In addition, VEGF gene family members play a key role in physiological and pathological angiogenesis. VEGF-A and its receptors have different functions including pro-angiogenic and vascular permeability activity in tumor angiogenesis. The other type VEGF-B has a variety of functions under pathological conditions such as cancerogenesis [10]. For this reason, it is important to reveal the relationship among these three molecules in colorectal adenocarcinoma.

The second point is that pathological inflammation is also a considerable mechanism in cases of cancer. In this occasion, we also include that IL-17A expression besides these EDIL3/DEL1 and VEGFA/B gene mutations and expression analysis in silico. Furthermore, the recruitment of neutrophils to the tumor microenvironment is strongly regulated to mediate patient protection, thereby minimizing chronic inflammation. The CD4+ T helper cell-derived cytokine IL-17A has been well characterized by maintaining the influx of leukocytes in the pathological inflammatory site. This cytokine is produced by activated and memory T cells and induces the production of other proinflammatory cytokines (TNF, IL-1) and some chemokines [11,12]. Although IL-17 plays an important role in inflammation and autoimmunity for host defense mechanisms, this cytokine has received considerable attention for cancer in recent years [11]. The information obtained from in vivo, in vitro, and clinical studies suggests that IL-17 is usually favorable for the tumorigenesis but sometimes has an adverse function in cancer pathogenesis [11-14]. The effects of this cytokine will vary with different tumors that may be associated with angiogenesis and inflammatory for the microenvironment. We will need to clear the mechanism of cancer pathogenesis due to this contradictory effect. In particular, IL-17A and its receptors are expressed in different tumor types. However, tumor development, progression, and role in response to therapeutic regimens are unclear.

This study aimed to clarify the role of EDIL3/DEL1 in colorectal cancer. To that end, we evaluated that the expression patterns and mutation profiles of EDIL3, VEGFA/B, and IL-17A molecules in colorectal cancer, the underlying correlation of these molecules in colorectal cancer, and predicted the mechanism of EDIL3 mediated functions.

Material and Methods

Data collection

The colorectal cancer patient data set was obtained from the TCGA database. Demographic, clinical, and genetic data regarding the patient group are summarized in Table 1. The data used in our study were obtained from public database TCGA; therefore, ethical approval was not required.

Mutation Analysis

The cBio Cancer Genomics Portal (available at: <http://cbioportal.org>) is an open-access web platform tool for interactive exploration of multidimensional cancer genomics data sets based on The Cancer Genome Atlas (TCGA), currently providing access to data from more than 5,000 tumor samples from cancer studies [15]. To examine mutations in EDIL3/DEL1, IL17-A, VEGF-A, and VEGF-B genes in colorectal cancer samples of patients, the type of cancer of interest was selected on the web interface. The selected TCGA data set comprised of genome sequencing data of 594 colorectal cancer patients. Comprehensive mutation analyses were performed with the OncoPrint and Mutations using the mutation characteristics of the target genes obtained from the web interface of the cBio Cancer Genomics Portal. The OncoPrint provides an overview of genomic alterations in particular genes affecting particular individual samples.

In-Silico Analysis

To determine the possible pathogenicity of the identified mutations, we used the scores provided by the Polymorphism Phenotyping v2 (PolyPhen-2), Screening for Non-Acceptable Polymorphisms (SNAP), and the Catalog of Somatic Mutations in Cancer (COSMIC) databases.

PolyPhen-2 (Polymorphism Phenotyping v2; available at: <http://genetics.bwh.harvard.edu/pph2>) is an automatic tool for prediction of the possible impact of an amino acid substitution on the structure and function of a human protein using structural and comparative evolutionary considerations. PolyPhen-2 calculates the functional description of SNPs, maps coding SNPs to gene transcripts, extracts protein sequence annotations and structural characteristics and constructs conservation profiles. The program estimates the probability of the missense mutation being damaging based on a combination of all these features and provides both a qualitative prediction (probably damaging, possibly damaging, benign or unknown) with a score [16].

SNAP (available at: <https://www.rostlab.org/services/SNAP/>) is a machine learning device called "neural network". It distinguishes between effect and neutral variants/non-synonymous SNPs by taking a variety of sequences and variant features into account. SNAP comprises evolutionary constraints, structural features, and protein annotation information. The most important single feature for SNAP prediction is conservation in a family of related proteins as reflected by PSIC scores [17]. The respective

mutations in the SNAP program are considered neutral when a value is between (-) 100 and 0 is assumed. The respective mutation is considered affected when a value between 0 and 100 is assumed.

Finally, the score given by the COSMIC (the Catalogue of Somatic Mutations in Cancer; available at: <https://cancer.sanger.ac.uk/cosmic>) database was used to predict and verify the pathogenic effect of detected mutations [18]. Evolutionary conservation analyzes of the detected mutant amino acids were evaluated among different species via “Multiple sequence alignment” tool in the PolyPhen-2 software.

Gene Expression Analysis

GEPIA (Gene Expression Profiling Interactive Analysis; available at: <http://gepia.cancer-pku.cn/index.html>) is an advanced interactive network supporting normal and tumor tissue samples gene expression profiling and interactive analyses. GEPIA offers customizable features such as differentially expressed tumor/normal analysis from the TCGA and the GTEx (Genotype-Tissue Expression) databases [19]. The gene expression profiles of EDIL3, IL17-A, VEGF-A, and VEGF-B obtained from 275 colorectal cancer samples and 349 healthy tissue samples were analyzed using box plot charts. Also, Pearson correlation analyses were performed between EDIL3 gene expression levels and other study genes using the software.

Statistical Analysis

All statistical analyses were carried out on the GEPIA database. The Kaplan-Meier curves regarding overall survival were used. Low and high expression groups were compared using the log-rank test. The Pearson test was performed for correlation analyses using an online database. $P < 0.05$ was considered statistically significant.

Results

Results of mutation analysis

The genome sequencing data in 594 colorectal cancer patients were analyzed via the cBioPortal interface, aiming to identify genetic changes in EDIL3, IL17-A, VEGFA and VEGFB in colorectal cancer samples. Of these patients, 6.2% were found to have at least one genetic change (missense mutation, amplification, or deletion) in the EDIL3, IL17-A, VEGF-A, and VEGF-B. The detailed list of detected changes was shown in Table 2 in the studied genes based on the results of the mutation analysis.

Of the studied genes, EDIL3 was identified as the most frequently altered gene with a percentage change of 2.7% in colorectal cancer patients (Figure 1A). A total of 11 changes (1 nonsense and 10 missense mutations) were detected in EDIL3. The identified nonsense mutation at p. I289* causes early termination of the EDIL3 polypeptide, resulting in truncated protein generation.

All of the mutations detected in the IL17-A (4) are missense mutations. A total of 3 mutations (1 frameshift and 2 missense mutations) were detected in the VEGFA. In the VEGFB, 3 mutations (1 missense, 1 frameshift, and 1 splice site mutation) were detected. The p.N208Kfs*12 and p.K129Rfs*5 frameshift mutations were respectively detected in the VEGFA and VEGFB genes, resulting in early termination of polypeptides and truncated protein generation. Because the p.X125_splice site mutation in VEGFB is located on the first base of the splice site,

Table 1. Demographic, clinical and genetic data of patients with colorectal cancer

Characteristic	Patient data n: 594 (%)
Gender	
Male	312
Female	280
NA	2
Diagnosis age, years	
	66 (range, 31-90)
Race Category	
White	285 (48%)
Black or African American	64 (10.8%)
Asian	12 (2%)
NA	232 (3.4%)
Tumor Type	
Colon Adenocarcinoma	378 (63.6%)
Rectal Adenocarcinoma	155 (26.1%)
Mucinous Adenocarcinoma of the colon and rectum	61 (10.3%)
Sample Type	
Primary	594
Tumor Disease Anatomic Site	
Colon	436 (73.4%)
Rectum	152 (25.6%)
NA	3 (0.5%)
Overall Survival Status	
Living	471 (79.3%)
Deceased	120 (20.2%)
NA	
Metastasis Stage Code	
M0	440 (74.1 %)
MX	62 (10.4%)
M1	69 (11.6%)
M1a	11 (1.9%)
M1b	3 (0.5%)
NA	9 (1.5%)
Neoplasm Disease Stage Cancer Code	
Stage I	249 (47.6)
Stage II	54 (10.3%)
Stage III	126 (24 %)
Stage IV	83 (15.8)
NA	11 (2.1%)
Alteration Frequency in Colorectal Cancer	
	(Frequency %)
EDIL3 Mutation	2.7%
IL-17A Mutation	1%
VEGFA Mutation	1.7%
VEGFB Mutation	0.8 %

which is 100% preserved across species in the evolutionary process; this mutation is likely to cause deficiencies in the VEGFB expression. The schematic representation of the domain architecture of the proteins and mutations identified in the colorectal cancer tissue samples are shown in Figure 1B.

Results of in-silico analysis

The results of the PolyPhen-2 database program analysis revealed that 8 out of 11 mutations in EDIL3 had pathogenicity scores close to 1, indicating their pathogenic nature. Figure 2A presents the mutations with estimated pathogenicity scores of 1 in detail as revealed by the analysis performed with

Table 2. Mutations of the EDIL3, IL-17A, VEGF-A and VEGF-B genes in colorectal cancer patients.

No	Gene	Nt alteration	Accession Number	Alteration type	Localization	AA position	Previously determined disease/browser	Clinical significance		
								Poly-Phen2 (score)	SNAP (score)	COSMIC prediction (score)
C-1	EDIL3	c.160T>G	COSV56923498	Missense mutation	EGF domain	p.F54V	Colon Adenocarcinoma	Possibly Damaging (0,94)	Effect (74)	Pathogenic (score 0.97)
C-2	EDIL3	c.370G>A	COSV56898095	Missense mutation	EGF domain	p.E124D	Rectal Adenocarcinoma	Benign (0,04)	Neutral (-26)	Pathogenic (score 0.98)
C-3	EDIL3	c.370G>C	COSV56898095	Missense mutation	EGF domain	p.E126G	Mucinous Adenocarcinoma of the rectum	Benign (0,13)	Effect (23)	-
C-4	EDIL3	c.527C>A	COSV56892697	Missense mutation	F5-F8 Type C	p.A176D	Colon Adenocarcinoma	Probably Damaging (0,99)	Effect (76)	Pathogenic (score 0.99)
C-5	EDIL3	c.553G>A	COSV56897132	Missense mutation	F5-F8 Type C	p.G185R	Rectum Adenocarcinoma	Probably Damaging (1,00)	Effect (57)	Pathogenic (score 0.98)
C-6	EDIL3	c.583C>T	COSV56908131	Missense mutation	F5-F8 Type C	p.R195C	Rectum Adenocarcinoma	Probably Damaging (1,00)	Effect (60)	Pathogenic (score 0.97)
C-7	EDIL3	c.616A>G	rs1477118490	Missense mutation	F5-F8 Type C	p.T206A	GNOMAD	Probably Damaging (0,97)	Effect (61)	-
C-8	EDIL3	c.818G>A	COSV56905805	Missense mutation	F5-F8 Type C	p.G273E	Colon Adenocarcinoma	Probably Damaging (1,00)	Effect (70)	Pathogenic (score 0.97)
C-9	EDIL3	c.867A>G	rs754832124	Nonsense mutation	F5-F8 Type C	p.I289*	Colon Adenocarcinoma	Probably Damaging (0,96)	-	-
C-10	EDIL3	c.1076A>C	COSV56904816	Missense mutation	F5-F8 Type C	p.D359A	Colorectal Cancer	Probably Damaging (0,97)	Neutral (-7)	Pathogenic (score 0.99)
C-11	EDIL3	c.1228C>G	rs1197973626	Missense mutation	F5-F8 Type C	p.L410V	TOPmed	Benign (0,01)	Effect (74)	-
C-12	IL-17A	c.161G>A	COSV60684056 rs201890924	Missense mutation	Coding site	p.R54Q	Rectum Adenocarcinoma	Benign (0,02)	Effect (74)	Neutral (0,04)
C-13	IL-17A	c.107C>A	COSV60685413	Missense mutation	Coding site	p.S36Y	Colon Adenocarcinoma	Possibly Damaging (0,761)	Neutral (-5)	Neutral (0,08)
C-14	IL-17A	c.316G>A	COSV60684255	Missense mutation	IL17A domain	p.V106M	Colon Adenocarcinoma	Probably Damaging (0,99)	Neutral (-35)	Neutral (0,05)
C-15	IL-17A	c.340C>G	rs139375510	Missense mutation	IL17A domain	p.P114H	Colon Adenocarcinoma	Probably Damaging (0,99)	Effect (74)	-
C-16	VEGFA	c.596C>T	COSM1444777	Missense mutation	VEGF-C	p.199L	Colon Adenocarcinoma	Benign (0,31)	Neutral (-7)	-
C-17	VEGFA	c.416_417del	NA	Frame Shift	VEGF-C	p.N208Kfs*12	Colon Adenocarcinoma	Probably Damaging (0,96)	Effect (20)	-
C-18	VEGFA	c.428G>A	rs369593555 COSM296967	Missense mutation	VEGF-C	p.R212H/C	Mucinous Adenocarcinoma of the rectum	Probably Damaging (1,00)	-	-
C-19	VEGFB	c.324C>G	rs201075965	Missense mutation	PDGF	p.S108N	Colon Adenocarcinoma	Probably Damaging (0,94)	-	-
C-20	VEGFB	c.375A>C	COSV58537198	Splice site mutation	Splice Site Region	p.X125_splice	Colon Adenocarcinoma	Pathogenic (score 0.92)	-	-
C-21	VEGFB	c.385_386del	COSV58536837	Frame Shift	Coding site	p.K129Rfs*5	Mucinous Adenocarcinoma of the rectum	Probably Damaging	-	-

the PolyPhen-2 software (Figure 2A). The list of pathogenic characteristics detected in the studied genes is presented in detail in Table 2.

An extensive analysis was performed on the EDIL3 mutations as it was identified as the gene that underwent the highest percentage of changes. The multiple sequence alignment option was used in the PolyPhen-2 program and amino acid sequences affected by the identified mutations were compared across different species. The analysis revealed that the missense mutations of p.F54V, p.E124D, p.A174D, p.G185R, p.R195C,

p.T206D, p.G273E, p.I289*, and p.D359N caused alterations in the amino acids at critical sites; which have been conserved across a variety of species throughout the evolutionary process (Figure 2B).

Results of gene expression analysis

The m-RNA expression analysis was performed to determine whether the gene expression profiles of EDIL3/DEL1, IL-17, VEGF-A, and VEGF-B varied in colorectal cancer samples compared to healthy tissue samples. Our results demonstrated that EDIL3 and VEGF-B were significantly down-regulated in

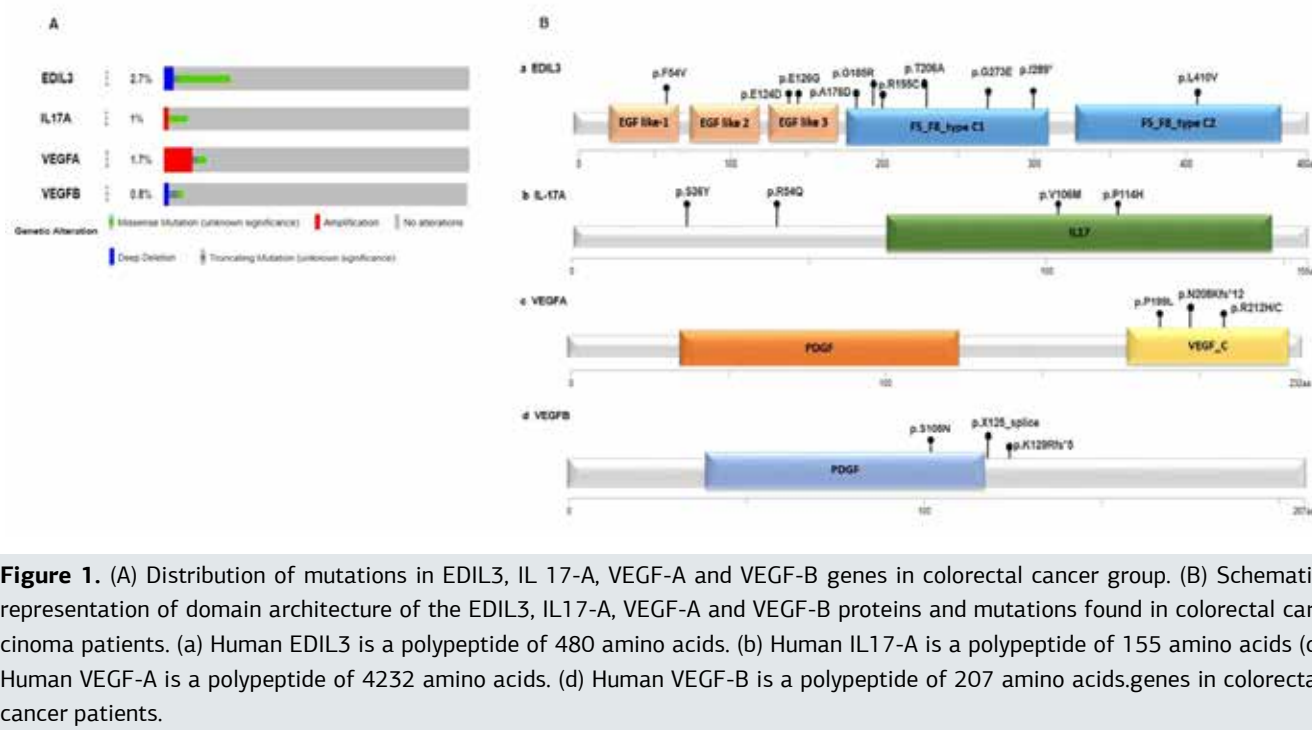


Figure 1. (A) Distribution of mutations in EDIL3, IL 17-A, VEGF-A and VEGF-B genes in colorectal cancer group. (B) Schematic representation of domain architecture of the EDIL3, IL17-A, VEGF-A and VEGF-B proteins and mutations found in colorectal carcinoma patients. (a) Human EDIL3 is a polypeptide of 480 amino acids. (b) Human IL17-A is a polypeptide of 155 amino acids (c) Human VEGF-A is a polypeptide of 4232 amino acids. (d) Human VEGF-B is a polypeptide of 207 amino acids. genes in colorectal cancer patients.

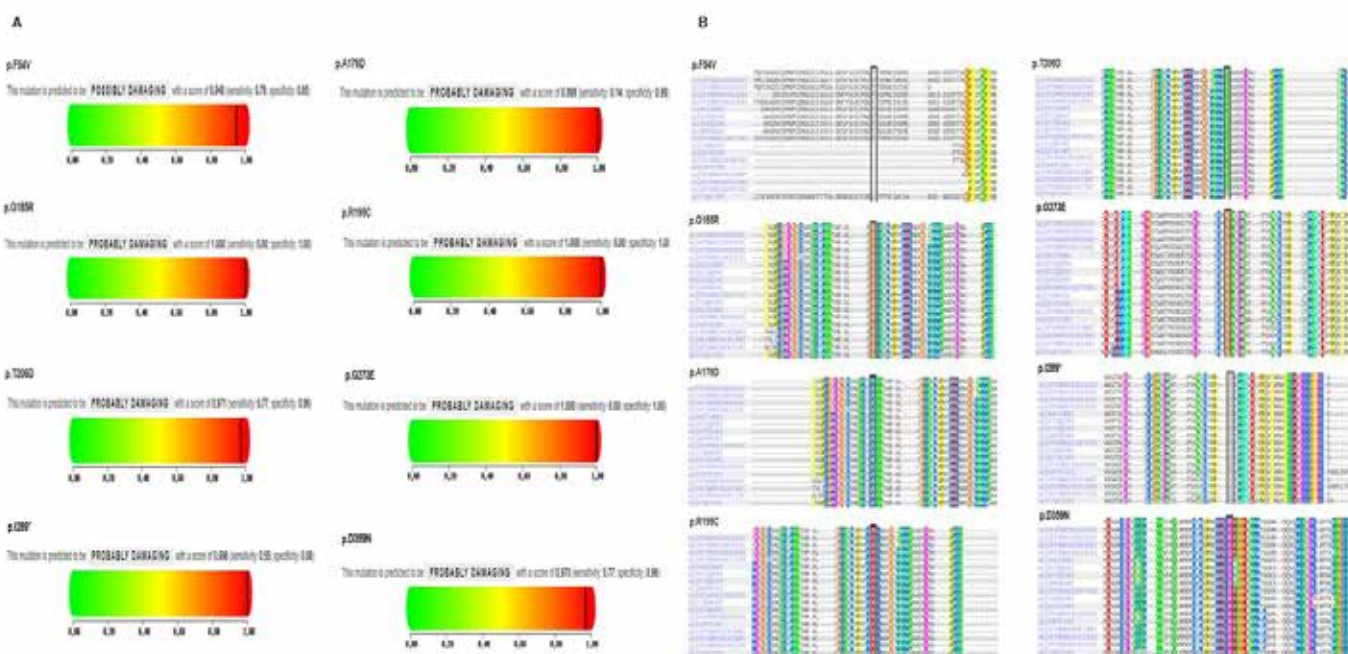


Figure 2. (A) Estimation of possible functional effects of mutations in EDIL3 gene with PolyPhen-2 program. (B) The evolutionary conservation analyses of the mutated amino acids in EDIL3 gene in the present study. The mutated amino acids were demonstrated. The detected mutant amino acids were evaluated among different species.

colorectal cancer patients compared to normal tissue samples (Figure 3A). No statistically significant changes in IL-17A and VEGFA were detected between the samples of tumor and healthy tissue. The correlation analysis of the individual results of IL17-A, VEGF-A, and VEGF-B m-RNA expression with the EDIL3 expression levels demonstrated that the IL17-A and EDIL3 expressions were inversely correlated but the correlation coefficient was low ($R = -0.17$, Figure 3B).

Discussion

This bioinformatic analysis showed mutation status and m-RNA expression levels of EDIL3, IL-17A, and VEGF in patient groups

with colorectal adenocarcinoma and healthy subjects. The associations of EDIL3, IL-17, and VEGF were also evaluated. Our study results showed that the level of EDIL3 was lower in the patient group compared with the controls. There was also a negative significant association between m-RNA expression levels of EDIL3 and IL-17. According to the literature, there is no direct comparative study about the association of EDIL3, IL-17, and VEGF molecules in colorectal adenocarcinoma samples. Most studies investigate the relationship between EDIL3 and IL-17 in periodontitis [20]. Cancer is the result of the accumulation of a series of genetic, epigenetic changes, and also inducers that lead to the deterioration of molecular

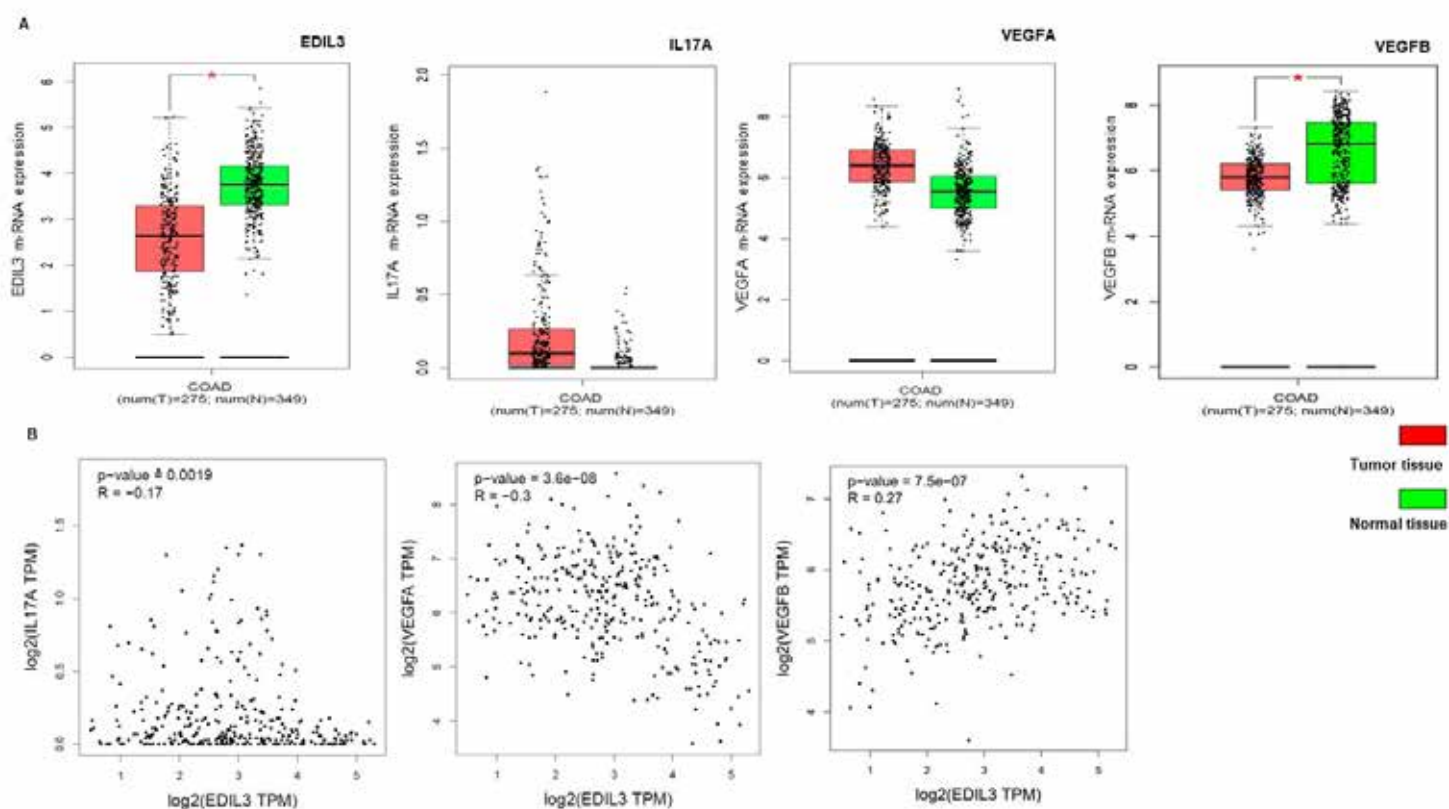


Figure 3. (A) Box plots of log₂ expression levels of EDIL3, IL-17A, VEGF-A and VEGF-B genes between healthy samples and colorectal cancer samples according to the TCGA data. The red and green boxes indicate colorectal cancer and normal tissues, respectively. Data were obtained from the GEPIA website, including 275 colorectal cancer samples and 349 healthy samples. * p<0.05. (B) Pearson correlation analyses of IL-17A, VEGF-A and VEGF-B m-RNA expressions with EDIL-3 expression.

mechanisms that control cell growth in normal tissue. Chronic inflammation especially is a causative factor that potentially leads to cancer development, and therefore its prevention can be a new therapeutic strategy for colorectal cancer [4,21]. It is known that EDIL3, which is associated with various inflammatory pathologies, has recently played a role in many cancers including hepatocellular carcinoma, colorectal cancer, bladder cancer, and pancreatic adenocarcinoma [5-9,22]. In addition, it has been reported that EDIL3 is defined as a new angiogenic factor and induces endothelial cell growth of tumor cells with high expression and regulates angiogenesis, which can promote the formation of new blood vessels [21]. However, the relationship between EDIL3 and cancer progression is not yet clear. Some studies have reported a decreased EDIL3 level in colorectal cancer and bladder carcinoma, while others have reported high EDIL3 expression in another type of cancer, such as hepatocellular carcinoma [7]. This inconsistency in the literature may result from tissue-specific transcriptional regulation, different tumor microenvironments, or different stages of cancer progression. There is no study in the literature that comprehensively examines the mutation profile in EDIL3 and associated genes and the effect of the association of the expression pattern on the pathogenesis of colorectal cancer. Therefore, in this study, we aimed to find evidence for its role in inflammatory pathology in colorectal cancer, as well as its role in inflammatory pathology in IL17-A, VEGFA/B, and its associated genes. It is obvious that angiogenesis and inflammatory environment affect the production of inflammatory cytokines

such as IL-17 and the main angiogenic marker VEGF. EDIL3 has an anti-inflammatory effect; the data have demonstrated that an experimental murine model with EDIL3 deficient was protected against periodontitis [20, 23]. Recent studies reported that the EDIL3 expression inhibits IL-17 expression periodontitis and increases VEGF expression in colorectal cancer [14,25].

In our study, the mutation profiles of EDIL3, IL17-A, VEGFA/B genes studied in 594 colorectal cancer patients who were included in the TCGA data sets were extensively analyzed, and 6.2% of the patient group carried the mutation and the greatest genetic change was observed. It was determined to be in EDIL3 (2.7%). Missense, splice site, and nonsense mutations due to reading frame changes were detected in the study genes, especially in the sequences encoding the major domains. The interspecies evolution analysis of the identified missense mutations of EDIL3, p.F54V, p.E124D, p.A174D, p.G185R, p.R195C, p.T206D, p.G273E, p.I289*, and p.D359N revealed that the preserved amino acids throughout the evolutionary process were affected while the functional analyses revealed a possibility for pathogenicity. These results suggest that they might have caused the generation of dysfunctional EDIL3 as it is a highly conserved protein in the evolutionary process. Furthermore, the mutations detected in each of these four genes are listed as somatic mutations in the COSMIC database. However, the mutations found in our study were reported to be involved in carcinogenesis for the first time because there are no studies in the literature indicating the role of EDIL3 mutations in the

pathogenesis of colorectal cancer. In addition, the frameshift mutations detected in our study would result in premature stops of amino acid synthesis in VEGFA/B proteins and as a consequence resembles typical loss-of-function mutations. Since recent studies have reported that VEGFA/B regulates angiogenic activities, these frameshift mutations detected in the present study might prevent angiogenic activities [24].

These data uncover a key role association of EDIL3, IL-17, and VEGF proteins comparing colon cancer patients with healthy controls. Here, we present that cytokines can function to promote tumor progression. One of the best characterized and major populations of cells of the immune response that produce IL-17 are CD4+ T cells of the Th17 subset of the helper T cells. Eskan and colleagues reported that EDIL3 inhibits IL-17 expression and suppresses the recruitment of polymorphonuclear cells (PMN) and the associated inflammation-mediated pathology in periodontitis [23]. In another way, IL-17 inhibits EDIL3 expression, which promotes the extravasation of neutrophils into the inflammation site. IL-17 acts in synergy with other proinflammatory cytokines such as TNF and IL-1 to induce extracellular matrix enzymes.

As a result of the bioinformatic analysis about expression profiles of EDIL3 and other related genes, our results confirm the inhibiting effect of EDIL3 upon IL-17A m-RNA expression level in the colorectal cancer patients compared to healthy subjects. Besides this, there was a positive correlation between EDIL3 and VEGFB. Similar to our results, Jiang and et al. reported a positive correlation between EDIL3 and angiogenesis [9]. However, Shen and et al. found that a negative correlation between EDIL3 and VEGF molecules [25].

Conclusion

We concluded that EDIL3 plays a dual role in colorectal cancer by blocking the movement of immune cells to inflammation sites, such as tumor microenvironment and their factors in cancer development and progression using an anti-inflammatory function. Conversely, this molecule regulates angiogenesis by increasing expression of VEGF molecules.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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